SUMMARY

Introduction:

Heart failure [HF] is a chronic, progressive disease with a prevalence rate up to 3-4% in the general population. Despite new therapeutic opportunities the morbidity and mortality of HF remain very high. Patients with end-stage, severe HF exhibit an annual mortality rate of nearly 50%.

Coexisting diseases often complicate the course of HF and have been recognized as one of the factors that worsen the prognosis of the HF. Renal failure is one of the most frequently comorbidity in HF, with a prevalence rate up to approximately 50%.

The pathophysiology of progressive multi-organ failure in HF is complex and not solely related to hemodynamic disorders. Better understanding of the causes that contribute to the reduction of estimated glomerular filtration rate [eGFR] is essential to develop new treatment strategies in order to improve the prognosis of patients.

Currently, numerous mechanisms have been postulated to be responsible for worsening renal function in the population of patients with HF. However, not all interactions are known. Researchers are still looking for factors whose modification could reduce the progression of renal failure and thus significantly improve prognosis of this group of patients.

Many analyses indicate a connection between coagulation disorders and endothelial dysfunction with renal failure. In HF endothelial dysfunction occurs in a variety of vascular beds and contributes renal microcirculatory impairment. To date, there are no analyses in the available literature investigating the association of thrombotic biomarkers with worsening renal function in the HF population. In the current dissertation, we assessed endothelial function and thrombotic biomarkers in a population of patients with HF and analyzed their

association with impaired renal function, together with other parameters that may influence eGFR reduction.

Aims of the study:

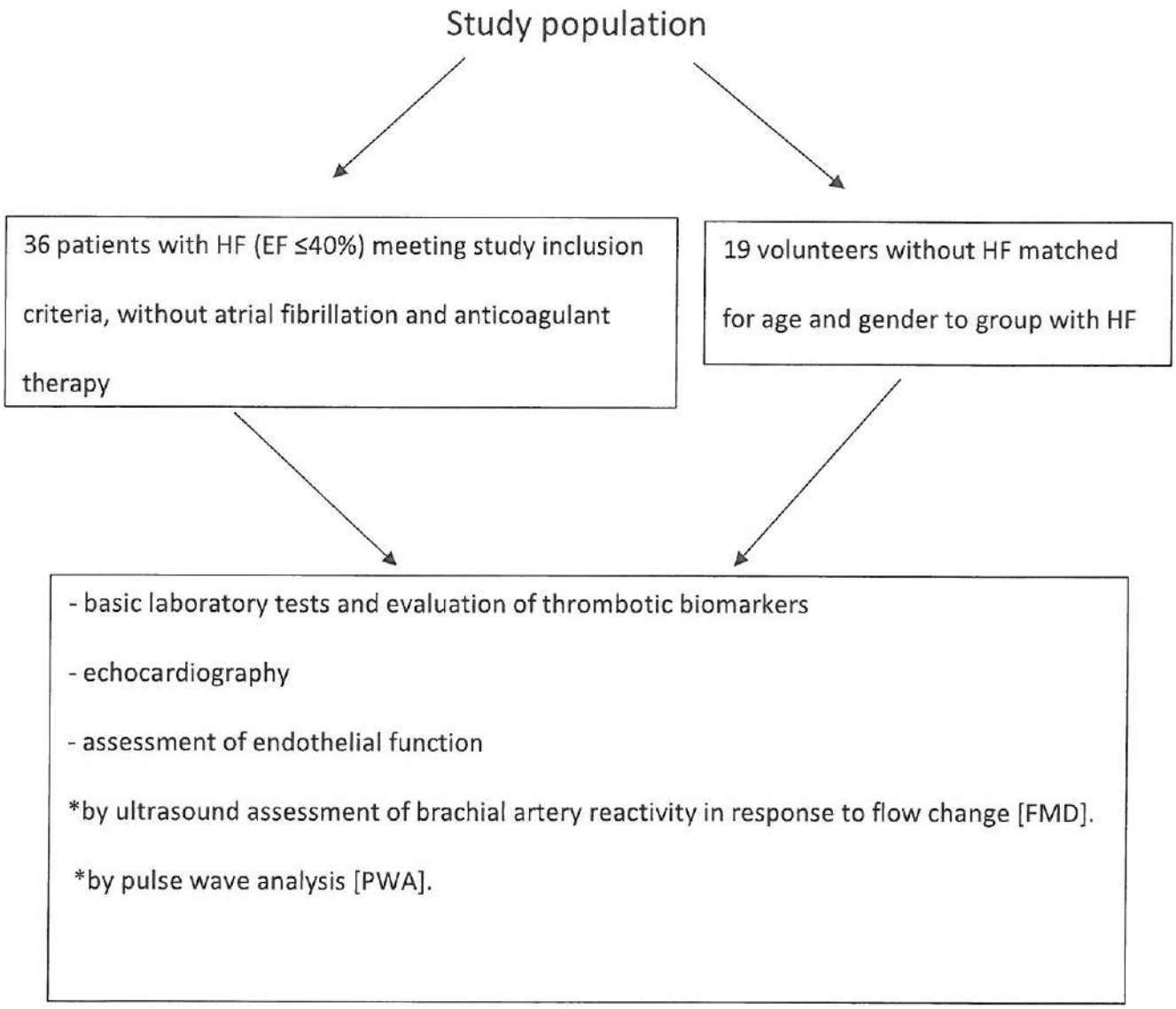
1. Evaluation of selected coagulation and endothelial dysfunction biomarkers in the population of HF patients and assessment of their correlation with biochemical, hemodynamic and echocardiographic parameters.
2. Comparison of selected coagulation biomarkers between HF study group and ~~a~~ control group without HF.
3. Identification of parameters associated with impaired renal function in the population of patients with HF.
4. Determination of a relationship between coagulation and endothelial dysfunction biomarkers and renal failure in the course of HF.

Materials and methods:

This is a cross-sectional study of subsequently electively admitted patients with HF (NYHA II-IV) with an ejection fraction (EF) ≤40% and sinus rhythm, hospitalized in the Department of Cardiology. A total of 36 patients were included in the study, along with 19 volunteers without HF (age- and gender-matched control group).

The inclusion criteria were as follows: diagnosed HF based on clinical symptoms and objective criterion of reduced systolic function (EF≤40%) determined by echocardiographic examination, sinus rhythm, patients above 18 years of age and absence of exclusion criteria. The exclusion criteria were: a history of cancer, dementia, atrial fibrillation, anticoagulant therapy in the 3 months preceding hospitalization, renal failure caused by primary renal

disease, infection in the 3 months prior to hospitalization that would require antibiotic therapy, and a lack of consent to participate in the study.



Results:

Characteristics of the study groups

The study population is a group of patients referred to the Department of Heart Failure and Transplantation as potential candidates for consideration of heart transplant eligibility. The study group with HF consisted of patients with a median age lower (=52 years) than in the standard HF population. In patients with heart failure, the predominant etiology of impaired systolic function was non-ischemic (n=21, 58.3%). Advanced HF (NYHA class III and IV) was presented in half of the study population of patients with HF (n=19, 52.8%). Men constituted the majority of the study population, more than 80% in the HF group (n=30, 83.3%). Blood pressure was higher in the control group (SBP in HF 108mmHg [102.5-118.3] vs 123.7mmHg [116.1-131.2] in control group, p<0.001) but it still remained within the normal range. Heart rate frequency was comparable in both groups and did not exceed 70 beats per minute (60n/min [56.7-68.0] in HF vs. 65.7n/min [62.0-69.7] in control group, p=0.28).

Patients with HF were mostly receiving optimal pharmacotherapy (assessed by the 2016 European Society of Cardiology guidelines), with 94.4% (n=34) of patients being treated with beta-blockers, 89% (n=32) of patients using ACEIs, 91.7% (n=33) using aldosterone antagonists (MRAs), and 89% (n=32) taking loop diuretics. None taking sacubitril/valsartan, sildenafil or anticoagulant therapy. All patients with ischemic etiology of heart failure (n=15) were on acetylsalicylic acid 75mg.

In tests assessing vascular reactivity, the study and control groups presented similar endothelium-dependent vascular reactivity assessed by FMD (5.0% [0.05-8.5] in HF, vs. 4.6% [2.3-6.7] in the control group, p=0.75), and also did not differ significantly in small vessel elasticity reflecting endothelial function as assessed by PWA (5.5 ml/mmHg x 100 [3.7-7.1] in HF vs 6.4 ml/mmHg x 100 [4.1-9.1] in control, p=0.09). On the other hand, large vessel

reactivity (large vessel compliance) was higher in the HF group, compared to the control group (18.9 ml/mmHg x10 [15.6-23] vs 13.9 ml/mmHg x10 [12.1-17.6], p=0.003). Furthermore, asymmetric dimethylarginine [ADMA] levels were significantly higher in the HF group (1.13µmol/l [0.86-1.4] vs 0.59µmol/l [0.5-0.7], p<0.001).

In addition, all parameters assessed by echocardiography indicating systolic dysfunction were significantly different compared to the normal values in the control group.

Laboratory results.

Among morphological parameters: red blood cell count (4.71 10^6/ul [4.4-4.9] vs 5.12 10^6/ul [5.0-5.3], p<0.001), hemoglobin concentration (14.4 g/dl [13.4-14.9] vs 15.7 g/dl [15.1-16.2], p<0, 001) and hematocrit (43.05% [40.2-45.4] vs 46.2% [44.4-47.9], p<0.001) were lower in the HF group, but were within reference values. In contrast, red blood cell variation rates were higher in the HF patient group (RDW SD 46.1 fL [43.9-50.1] vs 43.4 fL [41.7-45.2] p<0.001). In addition, higher creatinine levels (1.10 mg/dL [1.0-1.4] vs. 1.00 mg/dL [0.9-1.0], p=0.005) (with correspondingly lower glomerular filtration rate expressed by eGFR assessed by the MDRD formula) (70.3 ml/min/1.73m2 [54.9-85.3] vs. 82.9 ml/min/1.73m2 [78.2-90], p=0.004) were demonstrated in the HF group. International Normalized Ratio [INR] values were higher in the HF patient group, but did not exceed the normal values (1.07 [1.0-1.1] vs 0.98 [0.97-1.0], p<0.001). In contrast, other liver function parameters were within the reference values and there were no significant differences between the two groups (ALT 25 IU/l [18-43] vs 25 IU/l [21-34] p=0.81; total bilirubin 0.73 mg/dl [0.53-0.98] vs 0.65 mg/dl [0.47-0.79] p=0.18).

Thrombotic biomarkers analyzed.

Of the biomarkers of increased thrombotic activity studied, significantly higher levels of von Willenbrand factor [vWF] were observed in the HF patient group (1004.6 mU/ml [786.9-1242.3] vs 500.5 mU/ml [373.6-605.6], p<0.001). In contrast, the concentration of cleaved prothrombin fragments [F1+F2] was significantly higher in the control group (5.1 nmol/l [3.4-9.5] vs 11.2 nmol/l [8.3-11.9], p<0.001). In addition, soluble thrombomodulin [sTM] levels had borderline statistical significance and were higher in the HF group (12 ng/ml [9.3-16.9] vs 11.2 ng/ml [8.7-12.8], p=0.07). Moreover, among the factors with antithrombotic effects, only protein C activity was significantly reduced in the group with HF, compared to the control group (103% activity [92-119.5] vs 121% activity [98.7-129.5], p=0.04). The concentrations of other factors such as: thrombin-antithrombin complexes [TAT] (0.73 ng/l [0.20 - 3.68] vs 0.74 ng/l [0.34 - 3.64], p=0.19), plasminogen activator inhibitor type 1 [PAI-1] (43.5 ng/ml [30.97-50.5] vs 45, 0 ng/ml [41.8-49.3], p=0.2), tissue plasminogen activator [tPA] (23.8 U/l [15.7 - 32.0] vs 20.2 U/l [14.2 - 22.1] p=0.19), showed no significant differences between the study groups.

Association of specific categories of variables with renal function based on canonical analysis.

To examine the strength of the association of variables with renal function, given the large number of potential factors and the relatively small group size, canonical correlation was used to create five categories/indices for specific groups of variables: 1) laboratory parameters; 2) vascular reactivity; 3) thrombotic biomarkers (parameters); 4) hemodynamic parameters and 5) echocardiographic parameters.

The purpose of the canonical correlation was to demonstrate the relationship between the variables in each category and individual categories of variables and eGFR. Each category was a distinct group of similar factors. In each category, the association with individual variables and the dependent variable (eGFR) was evaluated, and the collinearity of the compared variables was assessed.

In each category, a group of factors most strongly associated with eGFR was selected using canonical correlation. It was shown that each of the created categories (indices) was significantly associated with renal function. The index of thrombotic biomarkers was the category of variables with the strongest association with eGFR (correlation coefficient = 0.7 $p<0.0001$). It included parameters such as sTM, vWF, protein C, and tPA.

Analysis of multiple variables to select predictive factors of renal function.

In a further step of the analyses, a multivariable linear regression model was created with the previously selected indexes of the studied parameters to assess which is most strongly associated with eGFR. This analysis showed that only thrombotic, hemodynamic and echocardiographic biomarkers were significantly associated with renal function. Of all the variables, the index of thrombotic biomarkers is most strongly associated with eGFR; it accounted for, as much as 48% of the variation in glomerular filtration rate. The other parameters played a lesser role.

A multivariable logistic regression model was then developed to assess which of the created indices was a predictor of impaired renal function. For this purpose, the entire study population was stratified according to the degree of renal dysfunction (eGFR <60ml/min/1.73m2). Of all variables analyzed, only male gender ($p=0.015$) and thrombotic

biomarker index (p=0.001) were independently associated with impaired renal function. The final model was highly predictive (p<0.001) and the area under the curve was 0.925.

Limitations of the study:

The main limitation of the study is the small, selected study group with HF, which makes it difficult to generalize conclusions and to clearly define clinical implications based on the results. Still, only selected thrombotic biomarkers were evaluated. Another aspect is the cross-sectional nature of the study, which makes it impossible to draw conclusions about the cause-and-effect relationship.

Conclusions:

1. Patients with HF exhibit endothelial activation and higher values of thrombotic biomarkers.
2. Of all the parameters analyzed, thrombotic biomarkers, including these directly related with endothelial dysfunction, are the strongest independent factors associated with eGFR variability.
3. Thrombotic biomarkers are independent predictors of renal failure in a representative sample of the population.
4. The determination of thrombotic biomarkers in patients with HF may potentially allow the identification of individuals at risk of renal failure. However, this needs to be confirmed in prospective studies.